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Meiotic Recombination: Too Much of a Good Thing?

Dispatch

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Proper chromosome segregation in meiosis requires the right number and distribution of crossovers. Recent work in budding yeast has revealed a meiosis-specific role for RecQ helicase in limiting crossovers, distinct from its known somatic role in maintaining genome stability.

The meiotic cell division is specialised to separate homologous chromosomes into the haploid germ cells. The accurate segregation of chromosomes in meiosis, and consequent reduction of ploidy from 2N to 1N, depends on their being sufficient, properly distibuted crossovers, or recombination sites. This process involves an intricate set of chromosomal and DNA interactions [1,2]. At a gross chromosomal level, the homologous chromosomes exhibit transient interactions that progress to pairing along their lengths. This culminates in the construction of a highly ordered proteinaceous structure called the synaptonemal complex, and concomitantly physical connections called chiasmata are formed. These provide the tension necessary for proper alignment of the homologues on the meiosis I metaphase spindle, and are revealed only after the synaptonemal complex has broken down and the chromosomes are pulled to opposite poles. Chiasmata are sites of DNA exchange — crossovers — between the homologues. They are non-randomly distributed as a result of a mysterious process termed interference, such that all chromosomes obtain at least one crossover necessary for proper disjunction of the homologues. In most organisms studied, too few crossovers or crossovers in the wrong places can lead to aneuploidy as a result of missegregation [3].

At the DNA level, meiotic recombination initiates as double-strand breaks which are processed through several steps. These include strand resection, one-ended single-strand invasion of homologous sequences, priming of DNA synthesis from the invasion, second-end capture and the formation of double Holliday junctions, followed by resolution as a crossover [4,5] (Figure 1). More double-strand breaks are made than result in crossovers, though non-crossover interactions still result in recombination — these can be detected genetically by non-Mendelian segregation patterns such as gene conversions [6]. At some stage, therefore, many events are processed into non-crossovers without maturing into fully ligated double Holliday junctions. The big questions that remain to be answered are when, and how, the decision to be, or not to be, a crossover is made.

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A number of genes have been identified in which mutations cause a reduction in crossovers and/or a loss of interference, resulting in the production of aneuploid gametes (reviewed in [1–3,5]). But until very recently, we knew of no genes where loss of function led to an increase in meiotic crossing over. This has changed with the recent publication in *Current Biology* of a paper by Rockmill *et al.* [7], who have shown that the budding yeast RecQ helicase SGS1 [8,9] has a meiosis-specific role in limiting the number of crossovers.

With a non-null truncation allele of *SGS1*, which does not suffer the extreme mitotic genomic instability of the null mutant [10], Rockmill *et al.* [7] observed a 50% increase in crossing over. In previous work on *sgs1* mutants, meiotic defects were noted but no increase in crossovers was observed [9]. This was probably because a smaller data set was examined, with less informative genetic intervals. Expression of a helicase mutant [11] appeared to complement meiotic defects resulting from a deletion of the gene. This contrasts with the new work of Rockmill *et al.* [7], showing that the helicase domain is important for the meiotic functions of Sgs1p. The difference between the behaviour of the truncated and helicase mutant forms of the protein likely reflects complex interactions between the domains of Sgs1p [10].

Cytological studies of the progression of meiosis and locations of meiotic proteins in wild-type and mutant cells can reveal potential roles of the RecQ helicases in meiosis. In a cytological analysis of *sgs1* mutants, Rockmill *et al.* [7] found that homologues appear to pair along their lengths earlier than in wild-type cells. This suggests that Sgs1p acts early during homologous chromosome interactions, prior to the appearance of double Holliday junctions and formation of mature synaptonemal complex. The authors propose that the role of Sgs1p is to remove or convert these initial DNA interactions into non-crossovers (Figures 1, 2) although the mechanism is unclear.

The stage of meiosis at which Sgs1p can be detected in yeast is not consistent with this proposed early role, however, as Sgs1p seems to colocalize with proteins thought to be at the sites of crossovers (Figure 2). This could be due to a number of factors, including a second role in crossover resolution. In mice, however, the timing and localisation of BLM — the human RecQ homologue associated with Bloom's Syndrome [12] — in relation to other meiotic proteins associated with early recombination events, such as Rad51, Dmc1 and Rpa [13–15], is consistent with RecQ helicase having an early role in the crossover/non-crossover decision (Figure 2).

RecQ helicases are highly conserved across all life and are important for maintaining genome stability in dividing eukaryotic cells [16]. Most work on this family of helicases has concentrated on their mitotic functions, which appear to involve interaction with topoisomerase III [8,16]. Mutations in many of the RecQ helicases result

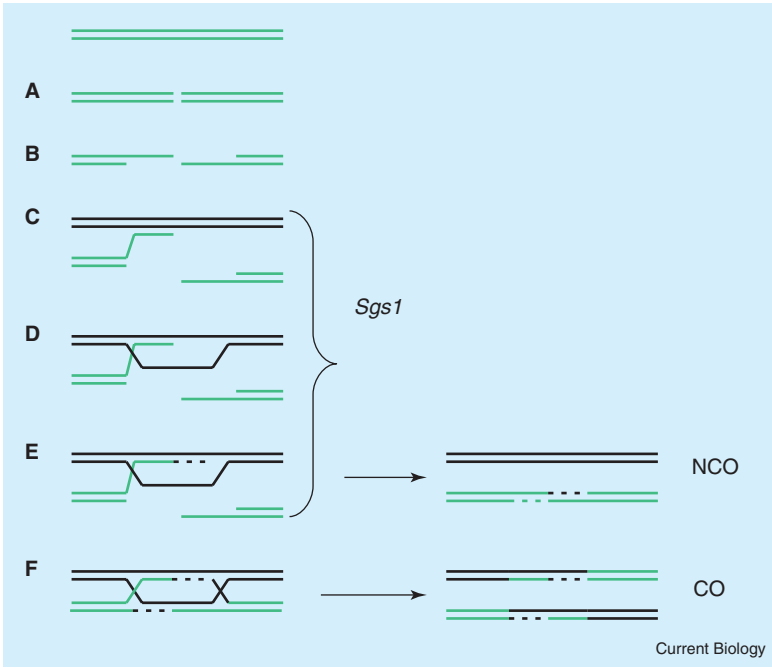

Figure 1. DNA processing and mechanics during meiosis in budding yeast.

(A) Meiotic recombination initiates with a double-strand break in one chromatid. (B) This is followed by resection of the 5′ ends leaving 3′ overhangs. (C) One single-stranded end interacts with a homologous chromatid, leading (D) to strand invasion and D-loop displacement. (E) This primes DNA synthesis. (F) The displaced D-loop 'captures' the other resected strand, forming a double Holliday junction and thereby a crossover (CO). Alternatively, unwinding of the newly synthesized DNA generates single-stranded DNA that anneals with the resected DNA resulting in a non-crossover (NCO). The decision of which double-strand breaks become crossovers is thought to occur early (C or D). Sgs1p is suggested to remove interactions not destined for crossovers, though its action must allow for repair of the double-strand break and so cannot be manifested until after DNA synthesis which is essential for repair (E). In the absence of Sgs1p, either some of the interactions that remain progress through to double Holliday junctions while others are eventually removed, or more interactions are 'marked' to become crossovers at the early decision point.

in hyper-recombination, chromosome loss and gross chromosomal rearrangements, as well as sensitivity to DNA damaging agents [8,9,16]. In some cases, a meiotic defect is also found. The new work [7], with earlier observations that yeast cells mutant in the sole RecQ homologue *SGS1* are sub-fertile [9,11], correlates well with observations on other species: male Bloom's syndrome patients are infertile, while females are sub-fertile [12]; and *Drosophila* mutants for the Bloom's homologue are infertile [17].

The somatic defects seen in these mutants are profound, making it difficult to know whether the infertility is an indirect result of the mitotic defects, or whether there are additional functions in meiosis for the

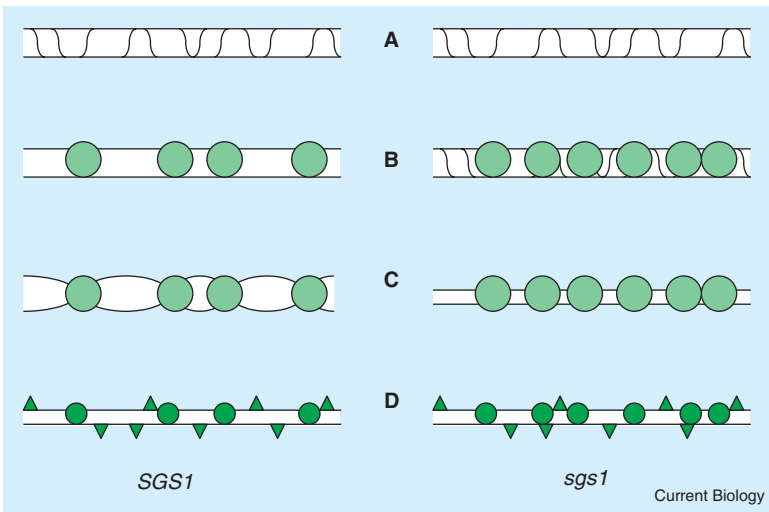

Figure 2. The progression of chromosome interactions to form a synaptonemal complex with mature crossovers.

The illustration compares a wild-type cell, on the left, with an *sgs1* mutant, on the right. (A) In both types of cell, at some point after resection of a double-strand break but before synaptonemal complex formation, DNA interactions between the two homologues occur at multiple sites. This may correspond with early DNA interactions seen in Figure 1. (B) A decision is made as to which of these interactions are destined to become crossovers and these are 'marked' (large circles) while Sgs1p is involved in the removal of the excess interactions. (C) The synaptonemal complex is propagated from these sites, leading (D) to a synaptonemal complex with mature crossover sites (small circles). At this stage, gene conversions (triangles) are also present. In an *sgs1* mutant cell, the numerous interactions are not removed early and more are 'marked' for crossing over (B). At the time when synaptonemal complex is propagated from the crossover sites the homologues are already in close proximity (C). At mature synaptonemal complex formation there are now 50% more mature crossovers along with the same level of gene conversions in non-crossovers. In mice, the timing and location of BLM, replication factors and recombination factors is consistent with BLM localising to interactions that are removed while not being located at the sites of recombination [15]. In yeast, Sgs1p appears to localise to the sites of mature synaptonemal complex propagation and crossover sites. This may be a detection problem, a spatial or temporal resolution problem, or the movement of Sgs1p after its action to those sites left. Perhaps Sgs1p has a role in the crossover resolution as well which has not yet been revealed genetically. Consistent with this idea and the cytological localisation, Sgs1p appears in a meiotic complex with Top3p, Mlh1p and Mlh3p that are involved in crossover production [20].

RecQ helicases. By comparing the effects of the *sgs1* truncation allele, which seems to cause little disruption of mitosis, with those of complete gene deletion, Rockmill *et al.* [7] showed that mitotic defects during growth are indeed responsible for much of the disruption of meiosis in the null mutant. But this cannot account for all of the problems with meiosis. The residual but significant meiotic defects caused by the truncation allele completely depend on the initiation of meiotic recombination, indicating that there is indeed a meiosis-specific role for Sgs1p. It is possible that the meiotic function for Sgs1p is also mediated via interaction with Top3p: *top3* mutants exhibit meiotic defects [18], though these are not precisely the same as those in *sgs1* mutants [7]. Indeed, Gilbertson and Stahl [19] proposed that a topoisomerase might resolve recombination intermediates into non-crossovers.

Can a surfeit of crossovers be bad? There is some evidence that too many crossovers may be associated with missegregation [3]. Yeast *sgs1* mutants exhibit a decrease in gamete viability which cannot be attributed to the mitotic defects, and might be the result of segregation problems caused by the excess crossovers. Indeed, Watt *et al.* [9] observed an increase in aneuploids in *sgs1* mutants. The new work of Rockmill *et al.* [7] shows that, not only are too few crossovers detrimental, but that perhaps too many can be harmful. RecQ mutations that do not cause mitotic genome instability may thus be a source of infertility. Future studies on Sgs1p in budding yeast should further light on precisely what the helicase does that is important in meiosis. Until then, all things even essential crossovers, should be taken in moderation.

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